Supplementary Information

Common variants associated with general and MMR vaccine-related febrile seizures

Bjarke Feenstra, Björn Pasternak, Frank Geller, Lisbeth Carstensen, Tongfei Wang, Fen Huang, Jennifer L. Eitson, Mads V. Hollegaard, Henrik Svanström, Mogens Vestergaard, David M. Hougaard, John W. Schoggins, Lily Yeh Jan, Mads Melbye, Anders Hviid

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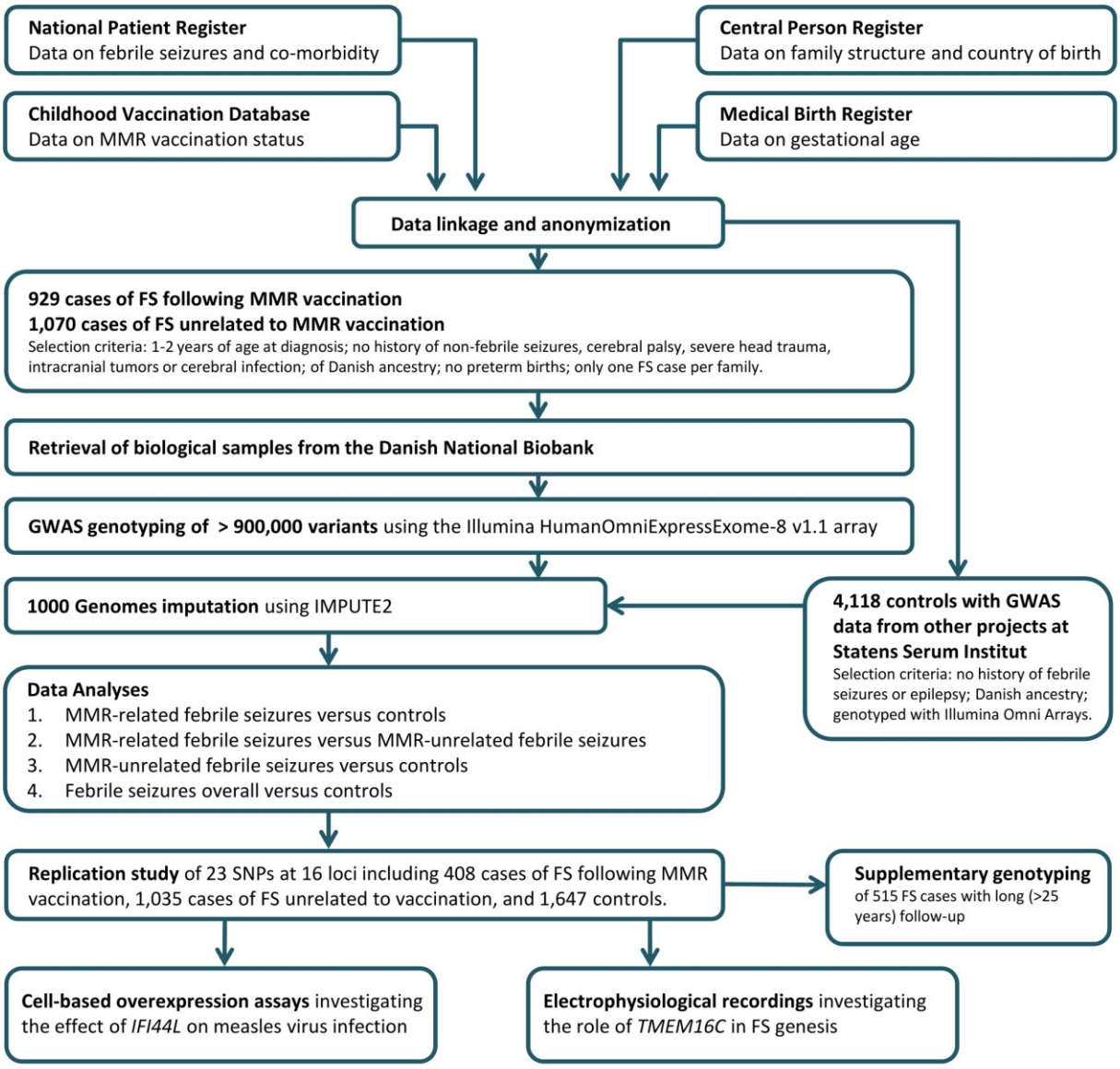
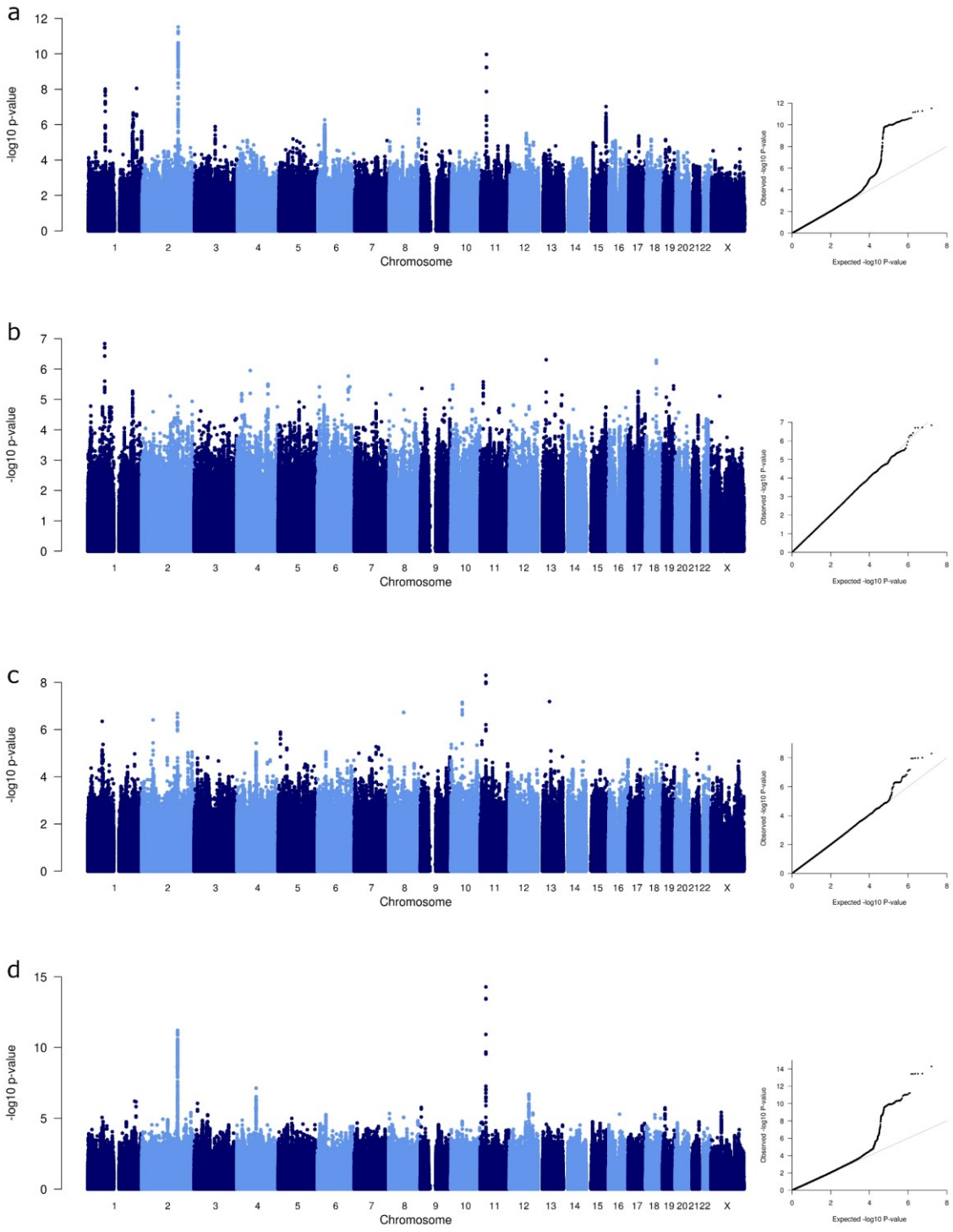
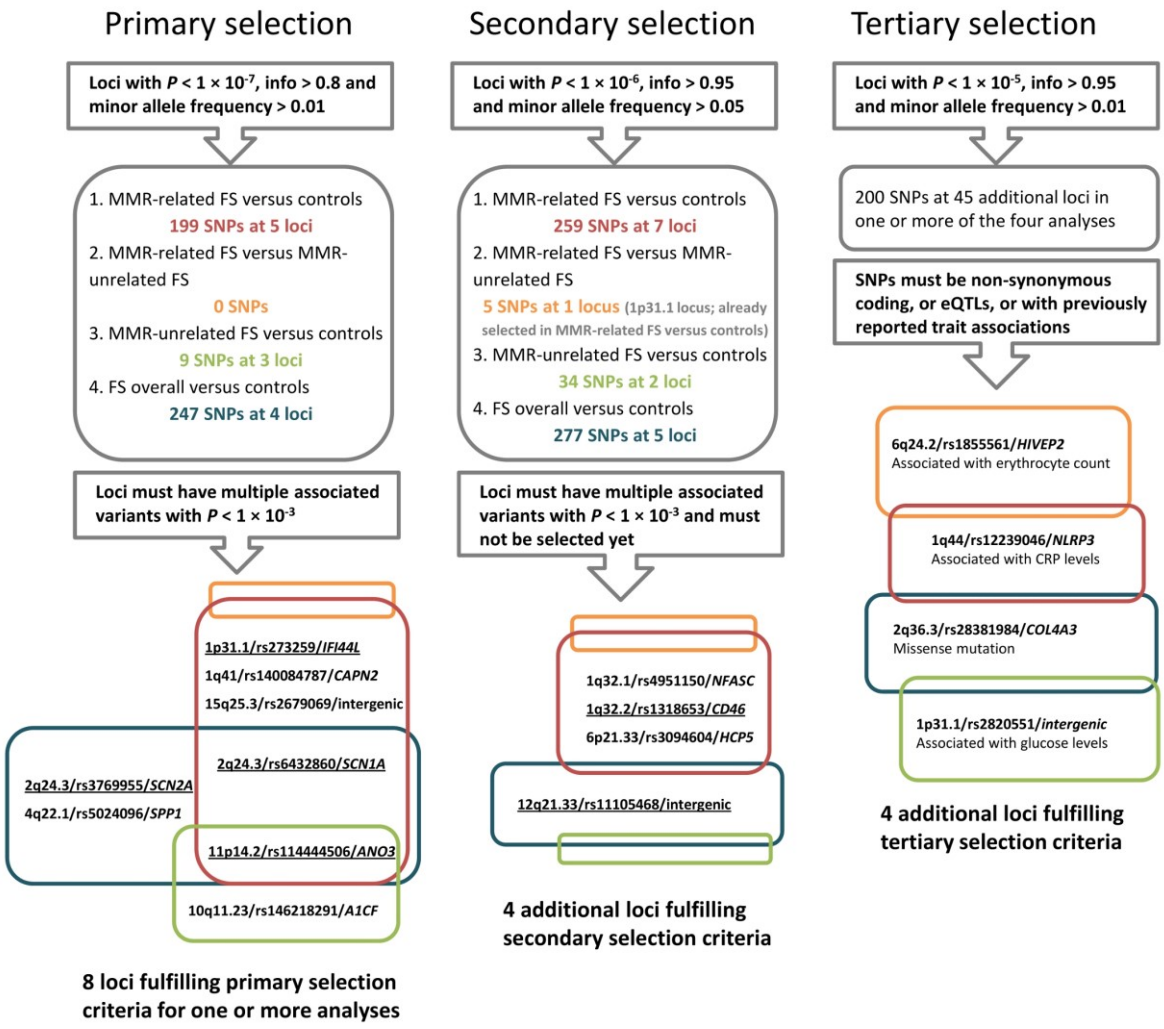


Supplementary Figure 1.
Study Design.



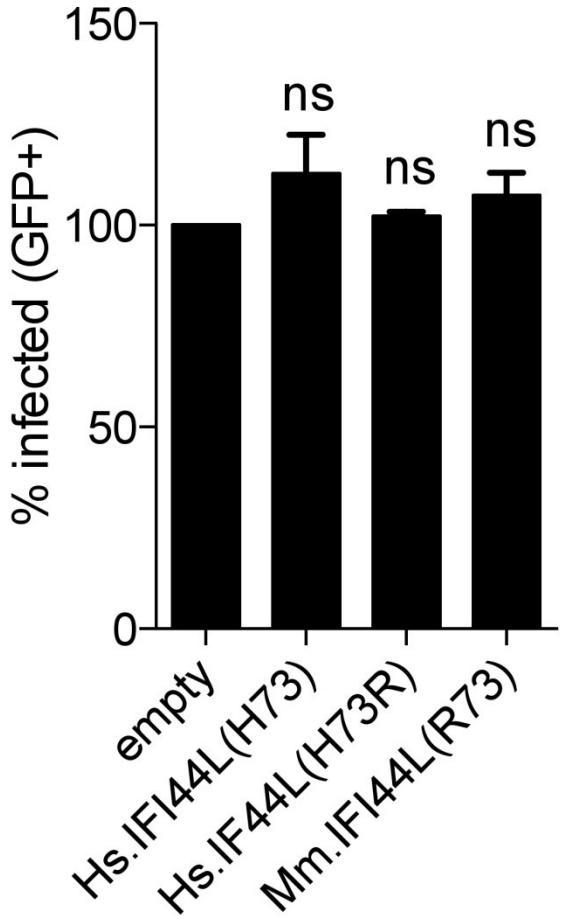
Supplementary Figure 2.

Manhattan plots of $-\log_{10} P$ values across the chromosomes (left panel) and corresponding quantile-quantile plots of observed versus expected $-\log_{10} P$ values (right panel). Observed P values were corrected by genomic control before plotting. (**a**) MMR-related febrile seizures versus controls. (**b**) MMR-related febrile seizures versus MMR-unrelated febrile seizures. (**c**) MMR-unrelated febrile seizures versus controls. (**d**) febrile seizures overall versus controls.



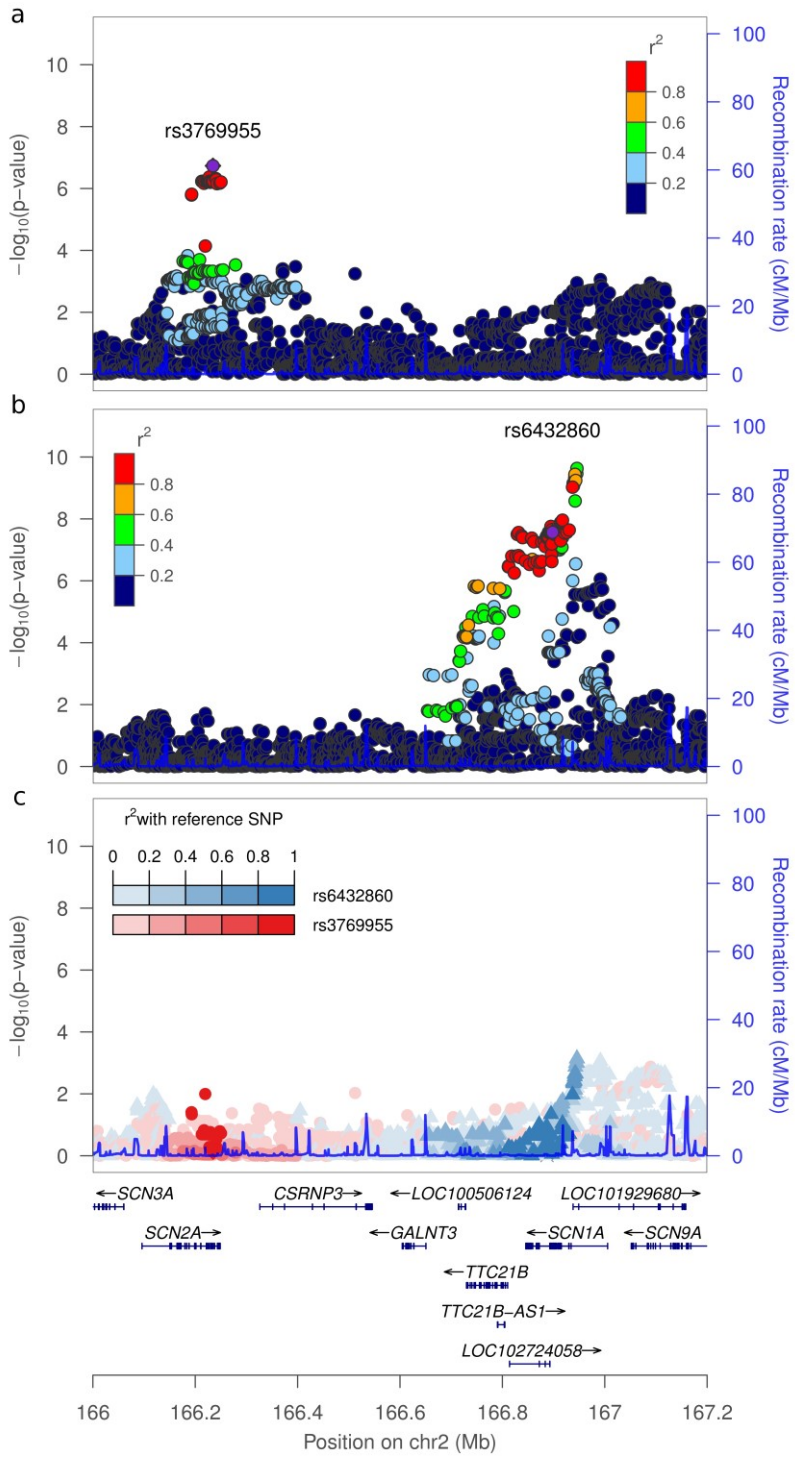
Supplementary Figure 3.

Criteria used to select SNPs for replication stage genotyping. SNP selection was carried out in three rounds with varying thresholds for *P* value, imputation quality (info), and minor allele frequency. Counts of SNPs and loci fulfilling the *P* value, info and minor allele frequency criteria are given for each of the four association analyses. It was further required that each locus should have at least one additional associated variant with $P < 1 \times 10^{-3}$ or that SNPs were non-synonymous coding, eQTLs, or had previously reported trait associations. A total of 16 loci were selected. Chromosomal band, top SNP, and nearest gene is written for each locus within a rounded rectangle colored red, orange, green, or blue according to the analysis on which the selection was based. Two loci were selected based on more than one of the four discovery analyses. Underlined loci are the ones that proved to be genome-wide significant in the combined analysis of discovery and replication data. For some loci, multiple SNPs were selected (see **Supplementary Table 2** for details).



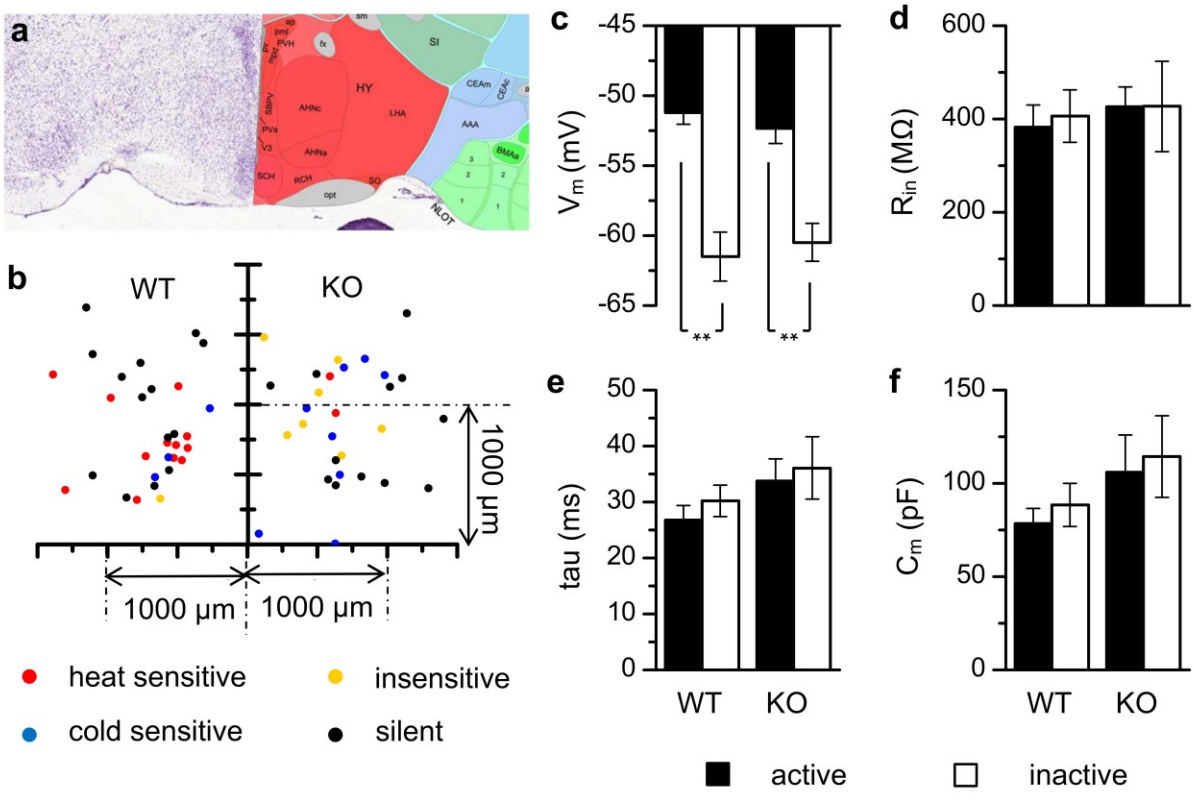
Supplementary Figure 4.

Infectivity of measles-GFP in *STAT1*$^{-/-}$ fibroblasts transduced with SCRPSY lentiviral vectors expressing an empty cassette or *IFI44L* variants (Human His73 variant; Human His73Arg variant; *Macaca mulatta* Arg73 variant). Virus infectivity was normalized to the empty control. Data represent the means of two independent experiments performed in triplicate. Statistical significance was assessed by t-test. ns, not significant. Error bars indicate standard deviations.



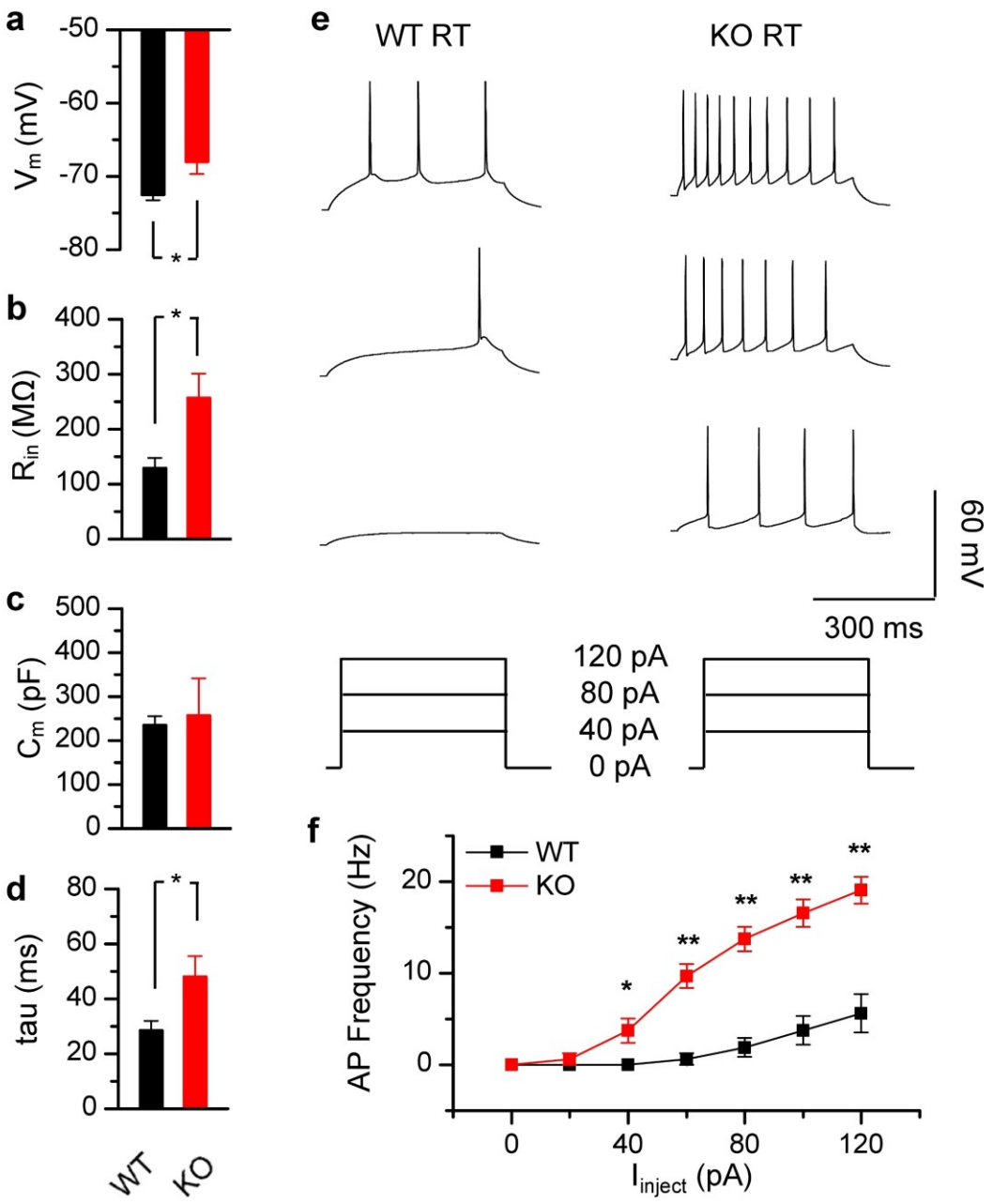
Supplementary Figure 5.

Discovery stage results from conditional analyses of febrile seizures overall versus controls. (**a–c)** Regional association plots for the larger 2q24.3 region, conditioning on (**a**) rs6432860, (**b**) rs3769955, and (**c**) both rs6432860 and rs3769955. SNPs position (x-axis) and disease association ($-\log_{10}$ *P* value; left y-axis) are shown, and the colors reflect linkage disequilibrium of each SNP with rs3769955 or rs6432860. Recombination rates are from HapMap (right y-axis).



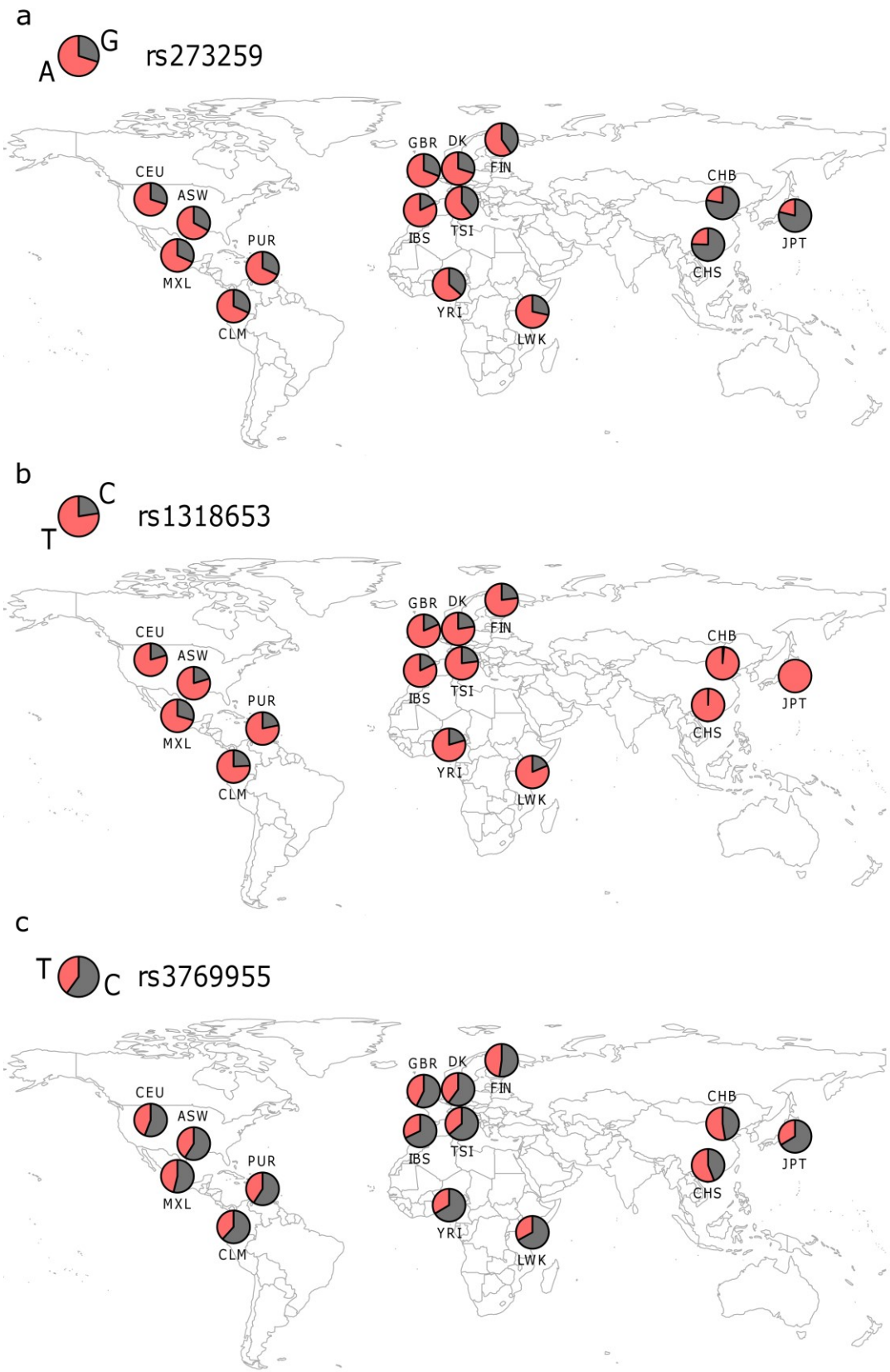
Supplementary Figure 6.

Distribution of the neurons recorded in the anterior hypothalamus (AHN) and their basic membrane properties. (**a**) Map of AHN from the Allen Mouse Brain Atlas[1] (http://atlas.brain-map.org/atlas?atlas=1&plate=100960284). (**b**) Distribution of every neuron from which electrophysiological data was obtained; x-axis, distance from the third ventricle; y-axis, distance from the optical chiasm. (**c**) Resting membrane potential (V_m), (**d**) input resistance (R_{in}), (**e**) time constant (τ), and (**f**) membrane capacitance (C_m) of AHN neurons at 36.5°C. Neurons from wild-type (WT) or *Tmem16C* knockout (KO) rats were classified into two categories: 1) active neurons that displayed spontaneous action potential (SAP) firing during the entire course of recordings, which correspond to the sum of heat-sensitive, cold-sensitive and temperature-insensitive neurons in **Figure 3**; and 2) inactive neurons that exhibited no SAP firing throughout the recordings, which is equivalent to the "silent" neurons in **Figure 3**. Two-way ANOVA was performed, and no difference was detected between WT and KO neurons ($P > 0.05$). The V_m shown in panel **C** is significantly different between active neurons and inactive neurons ($P < 0.01$, two-way ANOVA; **, $P < 0.01$, HSD-test). Error bars indicate s.e.m.



Supplementary Figure 7.

Hippocampal CA1 pyramidal neurons lacking *TMEM16C* exhibit hyperexcitability at room temperature. Basic membrane properties, namely (**a**) the resting membrane potential (V_m) ($P <$ 0.05, Student's t-test), (**b**) input resistance (R_{in}) ($P < 0.05$, Student's t-test), (**c**) membrane capacitance (C_m) ($P > 0.05$, Student's t-test) and (**d**) time constant (τ) ($P < 0.05$, Student's t-test) ($n = 9 – 11$). (**e**) Sample traces of the CA1 pyramidal neuronal responses to injections of 40 pA, 80 pA and 120 pA current, for wild-type (WT) and *Tmem16C* knockout (KO) neurons. (**f**) Current-steps elicit more action potentials in *Tmem16C* knockout neurons (red) than wild-type control (black) ($n = 9 – 11$; **, $P < 0.01$, *, $P < 0.05$, Student's t-test). Error bars indicate s.e.m.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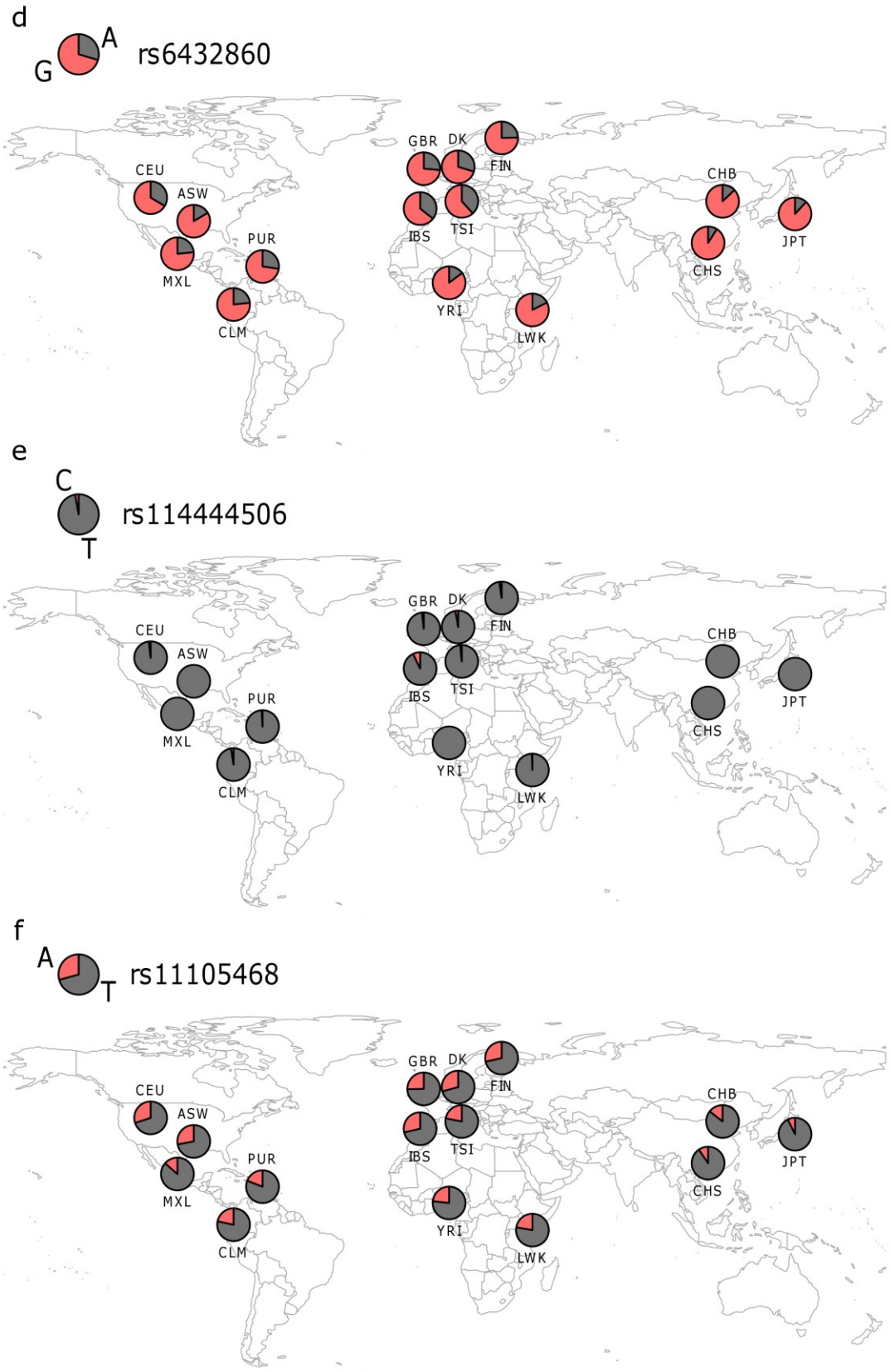
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Supplementary Figure 8.

Frequencies of risk (red) and protective (grey) alleles for the 6 genome-wide significant SNPs (**a–f**) in **Table 1** in the Danish controls (DK) and in 14 populations sampled by the 1000 Genomes Project. ASW, Americans of African ancestry in southwestern USA; CEU, Utah residents (CEPH) with northern and western European ancestry; CHB, Han Chinese in Beijing, China; CHS, Southern Han Chinese; CLM, Columbians from Medellin, Colombia; FIN, Finnish in Finland; GBR, British in England and Scotland; IBS, Iberian population in Spain; JPT, Japanese in Tokyo, Japan; LWK, Luhya in Webuye, Kenya; MXL, Mexican ancestry from Los Angeles USA; PUR, Puerto Ricans from Puerto Rico; TSI, Toscani in Italy; YRI, Yoruba in Ibadan, Nigeria. The 1000 Genomes Project allele frequencies were retrieved through the Ensembl browser (http://www.ensembl.org).

Supplementary Table 1.

Inclusion criteria and sample characteristics.

a	Discovery stage			Replication stage			
	MMR-related FS	MMR-unrelated FS	Controls	MMR-related FS	MMR-unrelated FS	Long follow-up FS	Controls
	(*n* = 929)	(*n* = 1,070)	(*n* = 4,118)	(*n* = 408)	(*n* = 1,035)	(*n* = 515)	(*n* = 1,647)
FS definition	Record of FS in the Danish National Patient Register; 1-2 years of age at index date of FS event.		–	|--------- As in discovery stage ----------|			–
Temporal relation to MMR vaccination	FS between 9 and 14 days after vaccination	None (FS ≥6 weeks after vaccination or without any vaccine exposure)	–	As in discovery stage	As in discovery stage	Information on vaccination not available	–
Additional criteria	No history of epilepsy or non-febrile seizure until 2 years of age (i.e. beyond FS diagnosis); No history of cerebral palsy, severe head trauma, intracranial tumor, bacterial meningitis, viral encephalitis or cerebral abscess up until the index diagnosis; Not born preterm; Danish ancestry; At most 1 case per family (no case in sibling or half-sibling).		No records of FS or epilepsy in the Danish National Patient Register; Danish ancestry.	As in discovery stage, but preterm births allowed	As in discovery stage	As in discovery stage and no diagnosis of epilepsy or non-febrile seizure until 25 years of age	As in discovery stage
Boys, No. (%)	471 (51)	601 (56)	2111 (51)	225 (55)	586 (57)	308 (60)	914 (55)
Year of birth, range	1991-2008	1991-2008	1957-2009	1991-2011	1991-2011	1986-1987	1998-2000
Year of birth, mean (SD)	1999 (5)	1999 (5)	1994 (9)	2002 (7)	2002 (7)	1987 (1)	1999 (1)
Gestational age, mean (SD), weeks	39.9 (1.2)	39.9 (1.3)	39.3 (2.5)	38.7 (2.7)	39.9 (1.3)	39.8 (1.3)	40.1 (1.5)

b

Medical conditions	**ICD-8 codes** (up through December 1993)	**ICD-10 codes** (from January 1, 1994)
Febrile seizures	78021	R560
Epilepsy and non-febrile seizures	345, 78020, 78029	G40-G41, R568
Bacterial meningitis, viral encephalitis, and cerebral abscess	013, 02701, 0949, 03609, 03610, 05201, 05302, 05403, 05501, 05601, 07202, 09049, 320, 322, 323, 324	A170A, A022C, A229C, A514B, A548D, A321, A390, A392, B003, B004, B050, B051, B060, B010, B011, B020, B021, B261, B262, G00-G09
Severe head trauma (skull fracture, cerebral contusion, and traumatic intracranial hemorrhage)	800, 801, 803, 851-854	S020-S021, S027-S029, S06, S070, S071
Cerebral palsy	34399, 34499	G80
Intracranial tumor (central nervous system and meninges)	191, 192, 19839, 19841, 19842, 225, 238	C70-72, C793, D32-33, D42-43

(a) Inclusion criteria and sample characteristics for discovery stage febrile seizure cases and controls, as well as replication stage febrile seizure cases and controls. (**b**) *International Statistical Classification of Diseases* (*ICD*) codes, eighth (*ICD*-8) or tenth (*ICD*-10) revision, used to define medical conditions. FS, febrile seizures.

Supplementary Table 2.

Association results for all SNPs included in the replication stage genotyping. Results with $P < 5\times10^{-8}$ are marked in bold. FS, febrile seizures. I^2, heterogeneity estimate. P_{het}, P value from Cochran Q test of heterogeneity. Alternating background color indicates different genetic loci. **Table supplied as an additional Excel spreadsheet (online)**.

Supplementary Table 3.

Association results for 48 SNPs with $P < 10^{-5}$ across the two loci distinctly associated with febrile seizures following MMR vaccination. The table is sorted by base pair position (NCBI build 37) and shows effect and alternative allele; effect allele frequency; info value from SNPTEST indicating imputation quality; odds ratio for association with febrile seizures following MMR vaccination; P value (after genomic control, λ=1.01 from discovery scan); indicator of whether the SNP was genotyped; squared Pearson correlation coefficient (r^2) of imputed SNP allele dosage to allele dosage for the top SNP at the locus (rs273259 or rs1318653); function class and gene name for SNPs in genes; PolyPhen-2 score and PolyPhen-2 and MutationTaster prediction for missense variants. Results for the two loci are separated by bold lines. **Table supplied as an additional Excel spreadsheet (online).**

Supplementary Table 4.

Association results for the SNPs achieving genome-wide significance in the febrile seizures overall versus controls in the main analysis, but here based on a replication group of febrile seizure cases with more than 25 years of follow-up without any records of epilepsy.

Chr	Position (bp)	SNP (effect allele/alter-native allele)	Sample Set	Freq Cases	Freq Controls	Number of Cases	Number of Controls	OR (95% CI)	*P* value	I^2	P_{het}
2	166234632	rs3769955 (T/C)	Discovery	0.459	0.400	1999	4118	1.28 (1.18-1.38)	7.9E-10		
			Replication*	0.440	0.417	512	1625	1.10 (0.95-1.26)	0.20		
			Combined			2511	5743	1.23 (1.15-1.32)	1.8E-09	70	0.07
2	166892788	rs2298771 (T/C)	Discovery	0.760	0.704	1999	4118	1.33 (1.22-1.45)	1.2E-10		
			Replication	0.737	0.710	514	1639	1.15 (0.98-1.34)	0.088		
			Combined			2513	5757	1.29 (1.19-1.39)	1.0E-10	62	0.11
2	166897864	rs6432860 (G/A)	Discovery	0.760	0.704	1999	4118	1.33 (1.22-1.45)	1.2E-10		
			Replication	0.739	0.709	513	1638	1.16 (0.99-1.36)	0.066		
			Combined			2512	5795	1.29 (1.20-1.39)	6.5E-11	55	0.14
2	166917694	rs1461201 (A/G)	Discovery	0.610	0.548	1999	4118	1.30 (1.20-1.41)	6.2E-11		
			Replication	0.589	0.550	513	1642	1.17 (1.02-1.35)	0.029		
			Combined			2512	5760	1.27 (1.19-1.36)	1.3E-11	39	0.20
2	166945367	rs13004083 (A/G)	Discovery	0.819	0.765	1999	4118	1.39 (1.27-1.53)	9.5E-12		
			Replication	0.803	0.771	513	1635	1.21 (1.02-1.45)	0.029		
			Combined			2512	5753	1.35 (1.24-1.47)	2.1E-12	45	0.18
11	26346831	rs114444506 (C/T)	Discovery	0.058	0.028	1999	4118	2.18 (1.79-2.64)	5.2E-15		
			Replication	0.058	0.028	514	1640	2.17 (1.56-3.03)	3.2E-06		
			Combined			2513	5758	2.18 (1.84-2.57)	1.4E-19	0	0.99
12	90328833	rs11105468 (A/T)	Discovery	0.340	0.292	1999	4118	1.24 (1.15-1.35)	2.0E-07		
			Replication	0.338	0.296	513	1640	1.22 (1.05-1.41)	0.010		
			Combined			2512	5758	1.24 (1.15-1.33)	6.6E-09	0	0.80

*The febrile seizure cases in this replication set are subjects with more than 25 years of follow-up without any records of epilepsy. Replication controls are the same as in the main study; I^2, heterogeneity estimate; P_{het}, *P* value from Cochran Q test of heterogeneity.

Supplementary Table 5.

Association results for the SNPs achieving genome-wide significance in the main analysis of febrile seizures overall versus controls, but here excluding all cases with epilepsy or unspecified convulsions recorded in the National Patient Register during subsequent follow-up after age 2.

Chr	Position (bp)	SNP (effect allele/alter-native allele)	Sample Set	Freq Cases	Freq Controls	Number of Cases	Number of Controls	OR (95% CI)	*P* value	I^2	P_{het}
2	166234632	rs3769955 (T/C)	Discovery	0.459	0.400	1907	4118	1.28 (1.18-1.38)	1.4E-09		
			Replication*	0.448	0.417	1387	1625	1.14 (1.03-1.26)	0.01		
			Combined			3294	5743	1.22 (1.15-1.30)	3.2E-10	67	0.08
2	166892788	rs2298771 (T/C)	Discovery	0.760	0.704	1907	4118	1.34 (1.22-1.46)	1.5E-10		
			Replication	0.764	0.710	1387	1639	1.31 (1.17-1.47)	3.7E-06		
			Combined			3294	5757	1.33 (1.24-1.42)	4.6E-15	0	0.80
2	166897864	rs6432860 (G/A)	Discovery	0.760	0.704	1907	4118	1.34 (1.22-1.46)	1.5E-10		
			Replication	0.765	0.709	1382	1638	1.33 (1.18-1.49)	1.3E-06		
			Combined			3289	5795	1.33 (1.24-1.43)	1.6E-15	0	0.94
2	166917694	rs1461201 (A/G)	Discovery	0.610	0.548	1907	4118	1.30 (1.20-1.41)	1.0E-10		
			Replication	0.597	0.550	1386	1642	1.21 (1.10-1.35)	2.2E-04		
			Combined			3293	5760	1.27 (1.19-1.35)	2.2E-13	12	0.28
2	166945367	rs13004083 (A/G)	Discovery	0.817	0.765	1907	4118	1.37 (1.25-1.52)	1.0E-10		
			Replication	0.809	0.771	1383	1635	1.25 (1.11-1.42)	3.6E-04		
			Combined			3290	5753	1.33 (1.23-1.43)	6.2E-13	23	0.25
11	26346831	rs114444506 (C/T)	Discovery	0.058	0.028	1907	4118	2.21 (1.82-2.69)	2.9E-15		
			Replication	0.053	0.028	1390	1640	1.97 (1.51-2.58)	8.0E-07		
			Combined			3297	5758	2.12 (1.81-2.49)	1.0E-20	0	0.50
12	90328833	rs11105468 (A/T)	Discovery	0.339	0.292	1907	4118	1.24 (1.14-1.34)	5.9E-07		
			Replication	0.345	0.296	1389	1640	1.26 (1.13-1.40)	3.9E-05		
			Combined			3296	5758	1.24 (1.16-1.33)	9.6E-11	0	0.83

*The median age at last follow-up for the febrile seizure cases was 15 years for the discovery set and 10 years for the replication set. I^2, heterogeneity estimate; P_{het}, *P* value from Cochran Q test of heterogeneity.

Supplementary Table 6

Association results for 347 SNPs with $P < 10^{-5}$ across the four loci associated with febrile seizures overall. The table is sorted by base pair position (NCBI build 37) and shows effect and alternative allele; effect allele frequency; info value from SNPTEST indicating imputation quality; odds ratio for association with febrile seizures overall; *P* value (after genomic control, λ=1.03 from discovery scan); indicator of whether the SNP was genotyped; squared Pearson correlation coefficient (r^2) of imputed SNP allele dosage to allele dosage for the top SNP at the locus (rs3769955, rs6432860, rs114444506, or rs11105468); function class and gene name for SNPs in genes; PolyPhen-2 score and PolyPhen-2 and MutationTaster prediction for missense variants. Results for the four loci are separated by bold lines. **Table supplied as an additional Excel spreadsheet (online)**.

Supplementary Table 7

Association results from the febrile seizures overall versus controls analysis for 9 SNPs showing genome-wide or suggestive association with serum magnesium concentration.

Chr	Position (bp)	SNP (effect allele/alter-native allele)	Info	Freq cases	Freq ctrls	OR (95% CI)	*P* value (discovery stage; association with febrile seizures overall)	Effect on serum magnesium concentration (mmol/L)	*P* value (association with serum magnesium levels)
1	155162067	rs4072037 (C/T)	0.933	0.429	0.422	1.03 (0.95-1.11)	0.47	-0.010	2.0E-36
2	176991779	rs2592394 (G/A)	1	0.696	0.696	1.00 (0.92-1.09)	0.97	0.004	4.6E-07
3	169100899	rs448378 (A/G)	0.994	0.526	0.520	1.03 (0.95-1.11)	0.50	0.004	1.3E-08
4	77412140	rs13146355 (A/G)	1	0.463	0.452	1.05 (0.97-1.13)	0.24	0.005	6.3E-13
9	77499796	rs11144134 (C/T)	0.970	0.083	0.079	1.05 (0.92-1.21)	0.46	0.011	8.2E-15
11	24678819	rs4561213 (G/T)	0.981	0.539	0.536	1.01 (0.94-1.09)	0.79	-0.004	2.6E-07
11	30760335	rs3925584 (C/T)	1	0.443	0.435	1.03 (0.95-1.11)	0.45	-0.006	5.2E-16
12	**90301679**	**rs7965584 (G/A)***	**0.998**	**0.337**	**0.293**	**1.23 (1.13-1.33)**	**8.0E-07**	**-0.007**	**1.1E-16**
16	68383047	rs7197653 (C/G)	0.954	0.156	0.156	1.00 (0.90-1.11)	0.94	-0.005	2.0E-06

*The top Mg^{2+} associated SNP at this locus, rs7965584, was not available in our imputed data. Instead febrile seizures overall association results are shown for rs10858938, a near-perfect proxy (r^2=0.96 in HapMap2); Reported effect estimates and *P* values for the association with magnesium levels in Meyer *et al*.[2] are shown in the last two columns; Info, Imputation quality (SNPTEST info); Freq, effect allele frequency.

Supplementary Table 8

Power analyses for the GWAS discovery scans at a significance threshold of $P < 1\times10^{-6}$.

		Allele frequency				
Analysis	**Odds ratio**	0.05	0.20	0.30	0.40	0.70
MMR+ *vs* controls	1.25	0%	15%	29%	37%	19%
	1.4	7%	86%	96%	98%	85%
	1.5	25%	99%	>99%	>99%	98%
	2.0	>99%	>99%	>99%	>99%	>99%
MMR+ *vs* MMR-	1.25	0%	3%	8%	11%	6%
	1.4	1%	43%	65%	74%	50%
	1.5	5%	80%	93%	96%	83%
	2.0	84%	>99%	>99%	>99%	>99%
MMR- *vs* controls	1.25	0%	21%	38%	47%	26%
	1.4	10%	92%	98%	99%	92%
	1.5	33%	>99%	>99%	>99%	99%
	2.0	>99%	>99%	>99%	>99%	>99%
All FS *vs* controls	1.25	2%	58%	80%	88%	69%
	1.4	33%	>99%	>99%	>99%	>99%
	1.5	72%	>99%	>99%	>99%	>99%
	2.0	>99%	>99%	>99%	>99%	>99%

Power estimates for the four discovery scans are shown at representative and relevant odds ratios (ORs) (OR = 1.25, OR = 1.4, OR = 1.5 and OR = 2.0) and risk allele frequencies (0.05, 0.20, 0.30, 0.40 and 0.70). ”MMR+” represents MMR-related febrile seizure cases and “MMR-“ represents MMR-unrelated febrile seizure cases. FS, febrile seizures.

Reference List

1. Lein,E.S. *et al.* Genome-wide atlas of gene expression in the adult mouse brain. *Nature* **445**, 168-176 (2007).

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